Amendments to the Specification

Please amend paragraph 0064 as follows:

[0064] The invention provides pharmaceutical compositions comprising a molecule of the invention, *e.g.*, a polypeptide comprising a variant Fc region, an immunoglobulin comprising a variant Fc region, a therapeutic antibody engineered in accordance with the invention, and a pharmaceutically acceptable carrier. The invention additionally provides pharmaceutical compositions further comprising one or more additional therapeutic agents, including but not limite**d** to anti-cancer agents, anti-inflammatory agents, immunomodulatory agents.

Please amend paragraph 0073 as follows:

[0073] As used herein, the term "immunomodulatory agent" and variations thereof refer to an agent that modulates a host's immune system. In certain embodiments, an immunomodulatory agent is an immunosuppressant agent. In certain other embodiments, an immunomodulatory agent is an immunostimulatory agent. ~~Immunomodatory~~ **Immunomodulatory** agents include, but are not limited to, small molecules, peptides, polypeptides, fusion proteins, antibodies, inorganic molecules, mimetic agents, and organic molecules.

Please amend paragraph 0098 as follows:

[0098] A kinetic screen was implemented to identify Fc region mutants with improved K_{off} rates for binding FcγRIIIA. A library of Fc region variants containing P396L mutation was incubated with 0.1 μM biotinylated FcγRIIIA-Linker-Avitag for one hour and then washed. Subsequently 0.8 ~~uM~~ **μM** unlabeled FcγRIIIA was ~~incubatd~~ **incubated** with the labeled yeast for different time points. Yeast was spun down and unlabeled FcγRIIIA was removed**[[,]].** Receptor bound ~~yease~~ **yeast** was stained with SA (streptavidin):PE (phycoerythrin) for FACS analysis.

Please amend paragraph 00105 as follows:

[00105] In a specific embodiment, the invention encompasses molecules comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild type Fc region, which variant Fc region only binds one FcγR, wherein said FcγR is FcγIIIA. In another specific embodiment, the invention encompasses molecules comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild type Fc region, which variant Fc region only binds one FcγR, wherein said FcγR is FcγRIIA. In yet another ~~**ambodiment**~~ **embodiment**, the invention encompasses molecules comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild type Fc region, which variant Fc region only binds one FcγR, wherein said FcγR is FcγRIIB. The invention particularly relates to the modification of human or humanized therapeutic antibodies (*e.g.*, tumor specific anti-angiogenic or anti-inflammatory monoclonal antibodies) for enhancing the efficacy of therapeutic antibodies by enhancing, for example, the effector function of the therapeutic antibodies, *e.g.*, enhancing ADCC.

Please amend paragraph 00110 as follows:

[00110] In a specific embodiment, the invention encompasses a molecule comprising a variant Fc region, wherein said variant Fc region does not have or are not solely a substitution at any of positions 268, 269, 270, 272, 276, 278, 283, 285, 286, 289, 292, 293, 301, 303, 305, 307, 309, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439 and does not have a histidine, glutamine, or tyrosine at position 280; a serine, glycine, threonine or tyrosine at position 290, a leucine or isoleucine at position 300; an asparagine at position 294, a proline at position 296; a proline, asparagine, aspartic acid, or valine at position 298; a lysine at position 295. In yet another preferred embodiment, the invention encompasses a molecule comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region, such that said molecule binds an FcγR with a reduced ~~**affnity**~~ **affinity** relative to a molecule comprising a wild-type Fc region

provided that said variant Fc region does not have or are not solely a substitution at any of positions 252, 254, 265, 268, 269, 270, 278, 289, 292, 293, 294, 295, 296, 298, 300, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, or 439. In yet another preferred embodiment, the invention encompasses a molecule comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region, such that said molecule binds an FcγR with an enhanced ~~affinity~~ **affinity** relative to a molecule comprising a wild-type Fc region provided that said variant Fc region does not have or are not solely a substitution at any of positions 280, 283, 285, 286, 290, 294, 295, 298, 300, 301, 305, 307, 309, 312, 315, 331, 333, 334, 337, 340, 360, 378, 398, or 430.

Please amend paragraph 00111 as follows:

[00111] In most preferred embodiments, the molecules of the invention with altered affinities for activating and/or inhibitory receptors having variant Fc regions, have one or more amino acid modifications, wherein said one or more amino acid modification is a substitution at position 288 with ~~asaparagine~~ **asparagine**, at position 330 with serine and at position 396 with leucine (MgFc10)(See Table 5); or a substitution at position 334 with glutamic acid, at position 359 with asparagine, and at position 366 with serine (MgFc13); or a substitution at position 316 with aspartic acid, at position 378 with valine, and at position 399 with glutamic acid (MgFc27); or a substitution at position 392 with threonine, and at position 396 with leucine (MgFc38); or a substitution at position 221 with glutamic acid, at position 270 with glutamic acid, at position 308 with alanine, at position 311 with histidine, at position 396 with leucine, and at position 402 with aspartic acid (MgFc42); or a substitution at position 240 with alanine, and at position 396 with leucine (MgFc52); or a substitution at position 410 with histidine, and at position 396 with leucine (MgFc53); or a substitution at position 243 with leucine, at position 305 with isoleucine, at position 378 with aspartic acid, at position 404 with serine, and at position 396 with leucine (MgFc54); or a substitution at position 255 with isoleucine, and at position 396 with leucine (MgFc55); or a substitution at position 370 with glutamic acid and at position 396 with leucine (MgFc59).

Please amend paragraph 00112 as follows:

[00112] In some embodiments, the invention encompasses molecules comprising a variant Fc region having an amino acid modification at one or more of the following positions: 185, 142, 192, 141, 132, 149, 133, 125, 162, 147, 119, 166, 251, 292, 290, 291, 252, 288, 268, 256, 262, 218, 214, 205,215, 247, 275, 202, 289, 258, 219, 279, 222, 246, 233, 246, 268, 244, 217, 253, 246, 224, 298, 280, 255, 218, 281, 284, 216, 223, 235, 221, 252, 241, 258, 227, 231, 215, 274, 287, 244, 229, 287, 291, 240, 281, 232, 269, 225, 246, 246, 293, 295, 248, 276, 268, 210, 288, 227, 221, 217, 261, 210, 242, 255, 240, 250, 247, 258, 246, 282, 219, 225, 270, 263, 272, 292, 233, 247, 254, 243, 347, 339, 392, 399, 301, 315, 383, 396, 385, 348, 333, 334, 310, 337, 371, 359, 366, 359, 379, 330, 318, 395, 319, 380, 305, 309, 335, 370, 378, 394, 386, 377, 358, 384, 397,372, 326, 320, 375, 327, 381, 354, 385, 335, 387, 353, 375, 383, 397, 345, 375, 389, 335, 394, 316, 399, 315, 394, 382, 390, 369, 377, 304, 323, 313, 388, 339, 317, 365, 367, 340, 311, 312, 398, 343, 352, 362, 303, 308, 327, 307, 344, 328, 393, 355, 360, 306, 361, 355, 415, 408, 409, 407, 424, 401, 402, 435, 421, 431, 441, 440, 435, 431, 442, 400, 422, 406, 411, 422, 433, 406, 423, 420, 412, 447, 443, 414, 433, 428, 446, 402, 419, 410, 404, 427, 417, 433, 436, 438, 416. Preferably such mutations result in molecules that have an altered affinity for an FcγR and/or has an altered ~~effecteor~~ **effector** cell mediated function as determined using methods disclosed and exemplified herein and known to one skilled in the art.

Please amend paragraph 00118 as follows:

[00118] The present invention is based, in part, on the identification of mutant human IgG1 heavy chain Fc regions, with altered affinities for different FcγR receptors, using a yeast display system. Accordingly, the invention relates to molecules, preferably polypeptides, and more preferably immunoglobulins (*e.g.*, antibodies), comprising a variant Fc region, having one or more amino acid modifications (*e.g.*, substitutions, but also including insertions or deletions) in one or more regions, which modifications alter the affinity of the variant Fc region for an FcγR. The affinities and binding properties of the molecules of the invention for an FcγR are initially determined using *in vitro* assays (biochemical or immunological based assays) known in the art for determining Fc-FcγR

interactions, *i.e.*, specific binding of an Fc region to an FcγR including but not limited to ELISA assay, surface plasmon resonance assay, immunoprecipitation assays (*See* Section 5.2.1). Preferably, the binding properties of the molecules of the invention are also characterized by in vitro functional assays for determining one or more FcγR mediator effector cell functions (*See* Section 5.2.6). In most preferred embodiments, the molecules of the invention have similar binding properties in in vivo models (such as those described and ~~**diselised**~~ **disclosed** herein) as those in in vitro based assays**.** However, the present invention does not exclude molecules of the invention that do not exhibit the desired phenotype in in vitro based assays but do exhibit the desired phenotype in vivo.

Please amend paragraph 00119 as follows:

[00119] The invention encompasses molecules comprising a variant Fc region, having one or more amino acid modifications (*e.g.*, substitutions) in one or more regions, wherein such modifications alter the affinity of the variant Fc region for an activating FcγR. In some embodiments, molecules of the invention comprise a variant Fc region, having one or more amino acid modifications (*e.g.*, substitutions) in one or more regions, which modifications increase the affinity of the variant Fc region for FcγRIIIA and/or FcγRIIA by at least 2-fold, relative to a comparable molecule comprising a wild-type Fc region. In another specific embodiment, molecules of the invention comprise a variant Fc region, having one or more amino acid modifications (*e.g.*, substitutions) in one or more regions, which modifications increase the affinity of the variant Fc region for FcγRIIIA and/or FcγRIIA by greater than 2 fold, relative to a comparable molecule comprising a wild-type Fc region. In other embodiments of the invention the one or more amino acid modifications increase the affinity of the variant Fc region for FcγRIIIA and/or FcγRIIA by at least 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, or 10-fold relative to a comparable molecule comprising a wild-type Fc region. In yet other embodiments of the invention the one or more amino acid modifications decrease the affinity of the variant Fc region for FcγRIIIA and/or FcγRIIA by at least 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, or 10-fold relative to a comparable molecule comprising a wild-type Fc region. Such fold increases are preferably determined by an ELISA or surface plasmon resonance assays.

In a specific embodiment, the one or more amino acid modifications do not include or are not solely a substitution at any one of positions 329, 331, or 322 with any amino acid. In certain embodiments, the one or more amino acid modification do not include or are not solely a substitution with any one of alanine at positions 256, 290, 298, 312, 333, 334, 359, 360, or 430; with lysine at position 330; with threonine at position 339; with methionine at position 320; with serine, asparagine, aspartic acid, or glutamic acid at position 326 with glutamine, glutamic acid, methionine, histidine, valine, or leucine at position 334. In another specific embodiment, the one or more amino acid modifications do not include or are not solely a substitution at any of positions 280, 290, 300, 294, or 295. In another more specific embodiment, the one or more amino acid modification does not include or are not solely a substitution at position 300 with leucine or isoleucine; at position 295 with lysine; at position 294 with asparagine; at position 298 with valine; aspartic acid proline, ~~**aspargine**~~ **asparagine**, or valine; at position 280 with histidine, glutamine or tyrosine; at position 290 with serine, glycine, ~~**theonine**~~ **threonine** or tyrosine.

Please amend paragraph 00122 as follows:

[00122] In a specific embodiment, the one or more amino acid modifications which increase the affinity of the variant Fc region comprise a substitution at position 347 with histidine, and at position 339 with valine; or a substitution at position 425 with isoleucine and at position 215 with phenylalanine; or a substitution at position 408 with isoleucine, at position 215 with isoleucine, and at position 125 with leucine; or a substitution at position 385 with glutamic acid and at position 247 with histidine; or a substitution at position 348 with methionine, at position 334 with asparagine, at position 275 with isoleucine, at position 202 with methionine, and at position 147 with threonine; or a substitution at position 275 with isoleucine, at position 334 with asparagine, and at position 348 with methionine; or a substitution at position 279 with leucine and at position 395 with serine; or a substitution at position 246 with threonine and at position 319 with phenylalanine; or a substitution at position 243 with isoleucine and at position 379 with leucine; or a substitution at position 243 with leucine, at position 255 with

leucine and at position 318 with lysine; or a substitution at position 334 with glutamic acid, at position 359 with asparagine, and at position 366 with serine; or a substitution at position 288 with methionine and at position 334 with glutamic acid; or a substitution at position 334 with glutamic acid and at position 380 with aspartic acid; or a substitution at position 256 with serine, at position 305 with isoleucine, at position 334 with glutamic acid and at position 390 with serine; or a substitution at position 335 with asparagine, at position 370 with glutamic acid, at position 378 with valine, at position 394 with methionine, and at position 424 with leucine; or a substitution at position 233 with aspartic acid and at position 334 with glutamic acid; or a substitution at position 334 with glutamic acid, at position 359 with asparagine, at position 366 with serine, and at position 386 with arginine; or a substitution at position 246 with ~~**threonineand**~~ **threonine and** at position 396 with histidine; or a substitution at position 268 with aspartic acid and at position 318 with aspartic acid; or a substitution at position 288 with asparagine, at position 330 with serine, and at position 396 with leucine; or a substitution at position 244 with histidine, at ~~**positon**~~ **position** 358 with methionine, at position 379 with methionine, at position 384 with lysine and at position 397 with methionine; or a substitution at position 217 with serine, at position 378 with valine, and at position 408 with arginine; or a substitution at position 247 with leucine, at position 253 with asparagine, and at position 334 with asparagine; or a substitution at position 246 with isoleucine, and at position 334 with asparagine; or a substitution at position 320 with glutamic acid and at position 326 with glutamic acid; or a substitution at position 375 with cysteine and at position 396 with leucine. Examples of other amino acid substitutions that results in an enhanced affinity for FcγRIIIA in vitro are disclosed below and summarized in Table 4.

Please amend paragraph 00127 as follows:

[00127] In some embodiments, the molecules of the invention have an altered ~~**affintiy**~~ **affinity** for FcγRIIIA and/or FcγRIIA as determined using *in vitro* assays (biochemical or immunological based assays) known in the art for determining Fc-FcγR interactions, *i.e.*, specific binding of an Fc region to an FcγR including but not limited to

ELISA assay, surface plasmon resonance assay, immunoprecipitation assays (*See* Section 5.2.1). Preferably, the binding properties of these molecules with altered affinities for activating FcγR receptors are also correlated to their activity as determined by in vitro functional assays for determining one or more FcγR mediator effector cell functions (*See* Section 5.2.6), *e.g.*, molecules with variant Fc regions with enhanced affinity for FcγRIIIA have an enhanced ADCC activity. In most preferred embodiments, the molecules of the invention that have an altered binding property for an activating Fc receptor, *e.g.*, FcγRIIIA in an in vitro assay also have an altered binding property in *in vivo* models (such as those described and disclosed herein). However, the present invention does not exclude molecules of the invention that do not exhibit an altered FcγR binding in in vitro based assays but do exhibit the desired phenotype *in vivo*.

Please amend paragraph 00128 as follows:

[00128] In a specific embodiment, the molecules of the invention comprise a variant Fc region, having one or more amino acid modifications (*i.e.*, substitutions) in one or more regions, which one or more modifications increase the affinity of the variant Fc region for FcγRIIIA and decreases the affinity of the variant Fc region for FcγRIIB, relative to a comparable molecule comprising a wild-type Fc region which binds FcγRIIIA and FcγRIIB with wild-type affinity. In a certain embodiment, the one or more amino acid modifications do not include or are not solely a substitution with alanine at any of positions 256, 298, 333, 334, 280, 290, 294, 298, or 296; or a substitution at position 298 with asparagine, valine, aspartic acid, or proline; or a substitution 290 with serine.. In certain amino embodiments, the one or more amino acid modifications increases the affinity of the variant Fc region for FcγRIIIA by at least 65%, at least 70%, at least 75%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 200%, at least 300%, at ~~**leat**~~ **least** 400% and decreases the affinity of the variant Fc region for FcγRIIB by at least 65%, at least 70%, at least 75%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 200%, at least 300%, at ~~**leat**~~ **least** 400%.

Please amend paragraph 00132 as follows:

[00132] The invention encompasses molecules comprising variant Fc regions, having one or more amino acid modifications, which modifications increase the affinity of the variant Fc region for FcγRIIIA and FcγRIIB by at least 65%, at least 70%, at least 75%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 200%, at least 300%, at ~~leat~~ **least** 400% and decreases the affinity of the variant Fc region for FcγRIIB by at least 65%, at least 70%, at least 75%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 200%, at least 300%, at ~~leat~~ **least** 400%. In a specific embodiment, the molecule of the invention comprising a variant Fc region with an enhanced affinity for FcγRIIIA and an enhanced affinity for FcγRIIB (as determined based on an ELISA assay and/or an ADCC based assay using ch-4-4-20 antibody carrying the variant Fc region as described herein) comprises a substitution at position 415 with isoleucine and at position 251 with phenylalanine; or a substitution at ~~positon~~ **position** 399 with glutamic acid, at position 292 with leucine, and at position 185 with methionine; or a substitution at position 408 with isoleucine, at position 215 with isoleucine, and at position 125 with leucine; or a substitution at position 385 with glutamic acid and at position 247 with histidine; or a substitution at position 348 with methionine, at position 334 with asparagine, at position 275 with isoleucine, at position 202 with methionine and at position 147 with threonine; or a substitution at position 246 with threonine and at position 396 with histidine; or a substitution at position 268 with aspartic acid and at position 318 with aspartic acid; or a substitution at position 288 with asparagine, at position 330 with serine and at position 396 with leucine; or a substitution at position 244 with histidine, at position 358 with methionine, at position 379 with methionine, at position 384 with lysine and at position 397 with methionine; or a substitution at position 217 with serine, at position 378 with valine, and at position 408 with arginine; or a substitution at position 247 with leucine, at position 253 with asparagine, and at position 334 with asparagine; or a substitution at position 246 with isoleucine and at position 334 with asparagine; or a substitution at ~~postion~~ **position** 320 with glutamic acid and at position 326 with glutamic acid; or a substitution at ~~position~~ **position** 375 with cysteine and at position 396 with leucine; or a substitution at position

343 with serine, at position 353 with leucine, at position 375 with isoleucine, at position 383 with asparagine; or a substitution at position 394 with methionine and at position 397 with methionine; or a substitution at position 216 with aspartic acid, at position 345 with lysine and at position 375 with isoleucine; or a substitution at position 288 with asparagine, at position 330 with serine, and at position 396 with leucine; or a ~~substition~~ **substitution** at position 247 with leucine and at position 389 with glycine; or a substitution at position 222 with asparagine, at position 335 with asparagine, at position 370 with glutamic acid, at position 378 with valine and at position 394 with methionine; or a substitution at position 316 with aspartic acid, at position 378 with valine and at position 399 with glutamic acid; or a substitution at position 315 with isoleucine, at position 379 with methionine, and at position 394 with methionine; or a substitution at position 290 with threonine and at position 371 with aspartic acid; or a substitution at position 247 with leucine and at position 398 with glutamine; or a substitution at position 326 with glutamine; at position 334 with glutamic acid, at position 359 with asparagine, and at position 366 with serine; or a substitution at position 247 with leucine and at ~~potisoon~~ **position** 377 with phenylalanine; or a substitution at position 378 with valine, at position 390 with isoleucine and at position 422 with isoleucine; or a substitution at position 326 with glutamic acid and at position 385 with glutamic acid; or a substitution at position 282 with glutamic acid, at position 369 with isoleucine and at position 406 with phenylalanine; or a substitution at position 397 with methionine; at position 411 with alanine and at position 415 with asparagine; or a substitution at position 223 with isoleucine, at position 256 with serine and at position 406 with phenylalanine; or a substitution at position 298 with asparagine and at position 407 with arginine; or a substitution at position 246 with arginine, at position 298 with asparagine, and at position 377 with phenylalanine; or a substitution at position 235 with proline, at position 382 with methionine, ~~atposition~~ **at position** 304 with glycine, at position 305 with isoleucine, and at position 323 with isoleucine; or a substitution at position 247 with leucine, at position 313 with ~~arginne~~ **arginine**, and at position 388 with glycine; or a substitution at position 221 with tyrosine, at position 252 with isoleucine, at position 330 with glycine, at position 339 with threonine, at position 359 with asparagine, at position 422 with

isoleucine, and at position 433 with leucine; or a substitution at position 258 with aspartic acid, and at position 384 with lysine; or a substitution at position 241 with leucine and at position 258 with glycine; or a substitution at position 370 with asparagine and ~~atposition~~ **at position** 440 with asparagine; or a substitution at position 317 with asparagine and a deletion at position 423; or a substitution ~~atposition~~ **at position** 243 with isoleucine, at position 379 with leucine and at position 420 with valine; or a substitution at ~~positon~~ **position** 227 with serine and at position 290 with glutamic acid; or a substitution at position 231 with valine, at position 386 with histidine, and at ~~postioon~~ **position** 412 with methionine; or a substitution at positions 215 with proline, at position 274 with asparagine, at position 287 with glycine, at position 334 with asparagine, at ~~positon~~ **position** 365 with valine and at position 396 with leucine; or a substitution at position 293 with valine, at position 295 with glutamic acid and at position 327 with threonine; or a substitution at position 319 with phenylalanine, at position 352 with leucine, and at position 396 with leucine; or a substitution at position 392 with threonine and at position 396 with leucine; at a substitution at position 268 with asparagine and at position 396 with leucine; or a substitution at position 290 with threonine, at position 390 with isoleucine, and at position 396 with leucine; or a substitution at position 326 with isoleucine and at position 396 with leucine; or a substitution at position 268 with aspartic acid and at position 396 with leucine; or a substitution at position 210 with methionine and at position 396 with leucine; or a substitution at position 358 with proline and at position 396 with leucine; or a substitution at position 288 with arginine, at position 307 with alanine, at position 344 with glutamic acid, and at position 396 with leucine; or a substitution at position 273 with isoleucine, at position 326 with glutamic acid, at position 328 with isoleucine and at position 396 with leucine; or a substitution at position 326 with isoleucine, at position 408 with asparagine and at position 396 with leucine; or a substitution at position 334 with asparagine and at position 396 with leucine; or a substitution at position 379 with methionine and at position 396 with leucine; or a substitution at position 227 with serine and at position 396 with leucine; or a substitution at position 217 with serine and at position 396 with leucine; or a substitution at position 261 with asparagine, at position 210 with methionine and at position 396 with leucine; or

a substitution at position 419 with histidine and at position 396 with leucine; or a substitution at position 370 ~~**woth**~~ **with** glutamic acid and at position 396 with leucine; or a substitution at position 242 with phenylalanine and at position 396 with leucine; or a substitution at position 255 with leucine and at position 396 with leucine; or a substitution at position 240 with alanine and at position 396 with leucine; or a substitution at position 250 with serine and at position 396 with leucine; or a substitution at position 247 with serine and at position 396 with leucine; or a substitution at position 410 with histidine and at position 396 with leucine; or a substitution at position 419 with leucine and at position 396 with leucine; or a substitution at position 427 with alanine and at position 396 with leucine; or a substitution at position 258 with aspartic acid and at position 396 with leucine; or a substitution at position 384 with lysine and at position 396 with leucine; or a substitution at position 323 with isoleucine and at position 396 with leucine; or a substitution at position 244 with histidine and at position 396 with leucine; or a substitution at position 305 with leucine and at position 396 with leucine; or a substitution at position 400 with phenylalanine and at position 396 with leucine; or a substitution at position 303 with isoleucine and at position 396 with leucine; or a substitution at position 243 with leucine, at position 305 with isoleucine, at position 378 with aspartic acid, at position 404 with serine and at position 396 with leucine; or a substitution at position 290 with glutamic acid, at position 369 with alanine, at position 393 with alanine and at position 396 with leucine; or a substitution at position 210 with asparagine, at position 222 with isoleucine, at position 320 with methionine and at position 396 with leucine; or a substitution at position 217 with serine, at position 305 with isoleucine, at position 309 with leucine, at position 390 with histidine and at position 396 with leucine; or a substitution at position 246 with asparagine; at position 419 with arginine and at position 396 with leucine; or a substitution at position 217 with alanine, at position 359 with alanine and at position 396 with leucine; or a substitution at position 215 with isoleucine, at position 290 with valine and at position 396 with leucine; or a substitution at position 275 with leucine, at position 362 with histidine, at position 384 with lysine and at position 396 with leucine; or a substitution at position 334 with asparagine; or a substitution at position 400 with proline; or a substitution at position 407

with isoleucine; or a substitution at position 372 with tyrosine; or a substitution at position 366 with asparagine; or a substitution at position 414 with asparagine; or a substitution at position 352 with leucine; or a substitution at position 225 with serine; or a substitution at position 377 with asparagine; or a substitution at position 248 with methionine.

Please amend paragraph 00133 as follows:

[00133] In some embodiments, the invention encompasses molecules comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region, which variant Fc region does not bind any FcγR, as determined by standard assays known in the art and disclosed herein, relative to a comparable molecule comprising the wild type Fc region. In a specific ~~**emobodiment**~~ **embodiment**, the one or more amino acid modifications which abolish binding to all FcγRs comprise a substitution at position 232 with serine and at position 304 with glycine; or a substitution at position 269 with lysine, at position 290 with asparagine, at position 311 with arginine, and at position 433 with tyrosine; or a substitution at position 252 with leucine; or a substitution at position 216 with aspartic acid, at position 334 with arginine, and at position 375 with isoleucine; or a substitution at position 247 with leucine and at position 406 with phenylalanine, or a substitution at position 335 with asparagine, at position 387 with serine, and at position 435 with glutamine; or a substitution at position 334 with glutamic acid, at position 380 with aspartic acid, and at position 446 with valine; or a substitution at position 303 with isoleucine, at position 369 with phenylalanine, and at position 428 with leucine; or a substitution at position 251 with phenylalanine and at position 372 with leucine; or a substitution at position 246 with glutamic acid, at position 284 with methionine and at ~~**postion**~~ **position** 308 with alanine; or a substitution at position 399 with glutamic acid and at position 402 with aspartic acid; or a substitution at position 399 with glutamic acid and at position 428 with leucine.

Please amend paragraph 00150 as follows:

[00150] In a specific embodiment, the invention encompasses engineering a humanized monoclonal antibody specific for Her2/neu protooncogene (*e.g.*, Ab4D5 humanized antibody as disclosed in Carter *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:4285-9) by modification (*e.g.*, substitution, insertion, deletion) of at least one amino acid residue which modification increases the affinity of the Fc ~~rgion~~ **region** for FcγRIIIA and/or FcγRIIA. In another specific embodiment, modification of the humanized Her2/neu monoclonal antibody may also further decrease the affinity of the Fc region for FcγRIIB. In yet another specific embodiment, the engineered humanized monoclonal antibodies specific for Her2/neu may further have an enhanced effector function as determined by standard assays known in the art and disclosed and exemplified herein.

Please amend paragraph 00151 as follows:

[00151] In another specific embodiment, the invention encompasses engineering a mouse human chimeric anti-CD20 monoclonal antibody, 2H7 by modification (*e.g.*, substitution, insertion, deletion) of at least one amino acid residue which modification increases the affinity of the Fc ~~rgion~~ **region** for FcγRIIIA and/or FcγRIIA. In another specific embodiment, modification of the anti-CD20 monoclonal antibody, 2H7 may also further decrease the affinity of the Fc region for FcγRIIB. In yet another specific embodiment, the engineered anti-CD20 monoclonal antibody, 2H7 may further have an enhanced effector function as determined by standard assays known in the art and disclosed and exemplified herein.

Please amend paragraph 00152 as follows:

[00152] In another specific embodiment, the invention encompasses engineering an anti-FcγRIIB antibody including but not limited to any of the antibodies disclosed in U.S. Provisional Application No. 60/403,266 filed on August 12, 2002 and U.S. Application No. 10/643,857 filed on August 14, 2003, having Attorney Docket No. 011183-010-999, by modification (*e.g.*, substitution, insertion, deletion) of at least one

amino acid residue which modification increases the affinity of the Fc ~~rgion~~ **region** for FcγRIIIA and/or FcγRIIA. Examples of anti-FcγRIIB antibodies that may be engineered in accordance with the methods of the invention are 2B6 monoclonal antibody having ATCC accession number PTA-4591 and 3H7 having ATCC accession number PTA-4592 (deposited at 10801 University Boulevard, Manassas, VA 02209-2011, which are incorporated herein by reference. In another specific embodiment, modification of the anti-FcγRIIB antibody may also further decrease the affinity of the Fc region for FcγRIIB. In yet another specific embodiment, the engineered anti-FcγRIIB antibody may further have an enhanced effector function as determined by standard assays known in the art and disclosed and exemplified herein. In a specific embodiment, the 2B6 monoclonal antibody comprises a modification at position 334 with glutamic acid, at position 359 with asparagine, and at position 366 with serine (MgFc13); or a substitution at position 316 with aspartic acid, at position 378 with valine, and at position 399 with glutamic acid (MgFc27); or a substitution at position 243 with isoleucine, at position 379 with leucine, and at position 420 with valine (MgFc29); or a substitution at ~~positon~~ **position** 392 with threonine and at position 396 with leucine (MgFc38); or a substitution at position 221 with glutamic acid, at ~~positon~~ **position** 270 with glutamic acid, at ~~positon~~ **position** 308 with alanine, at position 311 with histidine, at position 396 with leucine, and at position 402 with aspartic (MgFc42); or a substitution at position 410 with histidine, and at position 396 with leucine (MgFc53); or a substitution at position 243 with leucine, at position 305 with isoleucine, at position 378 with aspartic acid, at position 404 with serine, and at position 396 with leucine (MgFc54); or a substitution at position 255 with isoleucine, and at position 396 with leucine (MgFc55); or a substitution at position 370 with glutamic acid, and at position 396 with leucine (MgFc59) (See Table 5).

Please amend paragraph 00177 as follows:

[00177] Mutagenesis may be performed in accordance with any of the techniques known in the art including, but not limited to, synthesizing an oligonucleotide having one or more modifications within the sequence of the Fc region of an antibody or a

polypeptide comprising an Fc region (*e.g.*, the CH2 or CH3 domain) to be modified. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 30 to about 45 nucleotides or more in length is preferred, with about 10 to about 25 or more residues on both sides of the junction of the sequence being altered. A number of such primers introducing a variety of different mutations at one or more positions may be used to generate~~d~~ a library of mutants.

Please amend paragraph 00187 as follows:

[00187] In some embodiments, the invention encompasses identification of Fc mutants with altered FcγR affinities using affinity maturation methods which are known to those skilled in the art and encompassed herein. Briefly, affinity maturation creates novel alleles by randomly recombining individual mutations present in a mutant library, *see*, *e.g.*, Hawkins *et al.*, 1992, *J. Mol. Biol.* 226: 889-896; Stemmer *et al.*, 1994 *Nature*, 370: 389-91; both of which are incorporated herein by reference in their entireties. It has been used successfully to increase the affinity of antibodies, T cell receptors and other proteins. The invention encompasses using mutations that show increased FcγR binding as a baseline to construct new mutant libraries with enhanced phenotypes. Using the methods of the invention, a population of IgG1 Fc mutants enriched by yeast surface display for increased binding to an FcγR, *e.g.*, FcγRIIIA, may be selected. Following DNA preparation, Fc regions can be amplified by PCR using flanking primers that selectively amplify the mutated region of the Fc, which is about ~700 bp using methods known to one skilled in the art and exemplified or disclosed herein. Novel mutants can thus be constructed by reshuffling of mutations in the Fc region for example via DNAseI treatment of the amplified DNA and isolation of fragments using methods such as those disclosed by Stemmer *et al.*, 1994 *Proc. Natl. Acad. Sci. USA* 91: 10747-51, which is incorporated herein by reference in its entirety. Fragments can then be religated, PCR

amplified with nested primers and cloned into the yeast display vector, *e.g.*, pYD1 using methods known to one skilled in the art. The recombined library can then be reselected in the yeast Fc display screen. As the K_D decreases, below 10 nM, conditions can be established to allow for further increases in affinity based on the reduction of the off rate of the FcγRIIIA ligand from the Fc receptor using methods known in the art such as those disclosed in Boder *et al.*, 1998, *Biotechnol. Prog.* 14: 55-62, which is incorporated herein by reference in its entirety. The invention encompasses a kinetic screen of the yeast library. A kinetic screen may be established by labeling of the Fc displaying cells to saturation with a labeled ligand, e.g., a ~~fluoresenct~~ **fluorescent** ligand followed by incubation with an excess of non-labeled ligand for a predetermined period. After termination of the reaction by the addition of excess buffer (e.g., 1X PBS, 0.5 mg/ml BSA) cells will be analyzed by FACS and sort gates set for selection. After each round of enrichment individual mutants can be tested for fold increases in affinity and sequenced for diversity. The *in vitro* recombination process can be repeated. In some ~~embodiments~~ **embodiments**, the *in vitro* is repeated at least 3 times.

Please amend paragraph 00189 as follows:

[00189] The invention encompasses screening yeast libraries based on FcγRIIB depletion and FcγRIIIA selection so that Fc mutants are selected that not only have an enhanced affinity for FcγRIIIIA but also have a reduced affinity for FcγRIIB. Yeast libraries may be enriched for clones that have a reduced affinity for FcγRIIB by sequential depletion methods, for example, by incubating the yeast library with magnetic beads coated with FcγRIIB**.** FcγRIIB depletion is ~~preferrably~~ **preferably** carried out sequentially so that the library is enriched in clones that have a reduced ~~affinceity~~ **affinity** for FcγRIIB. In some embodiments, the FcγRIIB depletion step results in a population of cells so that only 30% , preferably only 10%, more preferably only 5%, most preferably less than 1% bind FcγRIIB**.** In some embodiments, FcγRIIB depletion is carried out in at least 3 cycles, at least 4 cycles, at least 6 cycles. The FcγRIIB depletion step is

~~preferrably~~ **preferably** combined with an FcγRIIIIA selection step, for example using FACS sorting so that Fc variants with an enhanced affinity for FcγRIIIIA are selected.

Please amend paragraph 00193 as follows:

[00193] According to a preferred embodiment of the invention, yeast cells are analyzed by fluorescence activated cell sorting (FACS). In most preferred embodiments, the FACS analysis of the yeast cells is done in an iterative manner, at least twice, at least three times, or at least 5 times. Between each round of selection cells are regrown and induced so the Fc regions are displayed on the ~~maxinum~~ **maximum** number of yeast cell surfaces. Although not intending to be bound by a particular mode of action, this iterative process helps enrich the population of the cells with a particular phenotype, *e.g.*, high binding to FcγRIIIA.

Please amend paragraph 00195 as follows:

[00195] The invention encompasses FACS screening of the mutant yeast library under equilibrium or kinetic conditions. When the screening is performed under equilibrium conditions, an excess of the yeast library carrying Fc mutants is incubated with FcγRIIIA, preferably labeled FcγRIIIA at a concentration 5-10 fold below the Kd, for at least one hour to allow binding of Fc mutants to FcγRIIIA under ~~equilbrium~~ **equilibrium** conditions. When the screening is performed under kinetic conditions, the mutant yeast library is incubated with labeled FcγRIIIA; the cells are then incubated with equimolar unlabeled FcγRIIIA for a pre-selected time, bound FcγRIIIA is then monitored.

Please amend paragraph 00247 as follows:

[00247] In some embodiments, the engineered antibodies of the invention are particularly effective in treating and/or preventing non-Hodgkin's lymphoma (NHL). The engineered antibodies of the invention are therapeutically more effective than current therapeutic regimens for NHL, including but not limited to chemotherapy, and immunotherapy using anti-CD20 mAb, Rituximab. The efficacy of anti-CD20

monoclonal antibodies however depends on the FcγR polymorphism of the subject (Carton *et al.*, 2002 *Blood,* 99: 754-8; Weng *et al.*, 2003 *J Clin Oncol.*21(21):3940-7 both of which are incorporated ~~**hrein**~~ **herein** by reference in their entireties). These receptors are expressed on the surface of the effector cells and mediate ADCC. High affinity alleles, of the low affinity activating receptors, improve the effector cells' ability to mediate ADCC. The methods of the invention allow engineering anti-CD20 antibodies harboring Fc mutations to enhance their affinity to FcγR on effector cells via their altered Fc domains. The engineered antibodies of the invention provide better immunotherapy reagents for patients regardless of their FcγR polymorphism.

Please amend paragraph 00273 as follows:

[00273] In certain embodiments, the one or more amino acid modifications, which increase the affinity of the variant Fc region for FcγRIIB but decrease the affinity of the variant Fc region for FcγRIIIA comprise a substitution at position 246 with threonine and at position 396 with histidine; or a substitution at position 268 with aspartic acid and at position 318 with aspartic acid; or a substitution at position 217 with serine, at position 378 with valine, and at position 408 with arginine; or a substitution at position 375 with cysteine and at position 396 with leucine; or a substitution at position 246 with ~~**isoleucine**~~ **isoleucine** and at position 334 with asparagine. In one embodiment, the one or more amino acid modifications, which increase the affinity of the variant Fc region for FcγRIIB but decrease the affinity of the variant Fc region for FcγRIIIA comprise a substitution at position 247 with leucine. In another embodiment, the one or more amino acid modification, which increases the affinity of the variant Fc region for FcγRIIB but decreases the affinity of the variant Fc region for FcγRIIIA comprise a substitution at position 372 with tyrosine. In yet another embodiment, the one or more amino acid modification, which increases the affinity of the variant Fc region for FcγRIIB but decreases the affinity of the variant Fc region for FcγRIIIA comprise a substitution at position 326 with glutamic acid. In one embodiment, the one or more amino acid modification, which increases the affinity of the variant Fc region for FcγRIIB but

decreases the affinity of the variant Fc region for FcγRIIIA comprise a substitution at position 224 with leucine.

Please amend paragraph 00395 as follows:

[00395] *Equilibrium screen:* An appropriate amount of cells was incubated while maintaining an ~~excesss~~ **excess** of ligand. For example, it is preferred to start with a number of cells needed to ensure 10-fold coverage of the library. For the first sort with a library containing 10^7 transformants, 10^8 cells should be used. In fact it is best to start with 10^9 cells to compensate for loss during the staining protocol.

Please amend paragraph 00404 as follows:

[00404] Table 10, summarizes the mutations that have been identified and their ~~correponding~~ **corresponding** binding characteristics to FcγRIIIA and FcγRIIB, as determined by both yeast display based assays and ELISA. In Table 6, the symbols represent the following: • corresponds to a 1-fold increase in affinity; + corresponds to a 50% increase in affinity; - corresponds to a 1-fold decrease in affinity; → corresponds to no change in affinity compared to a comparable molecule comprising a wild-type Fc region.

Please amend paragraph 00412 as follows:

[00412] ~~Additonal~~ **Additional** 4-4-20 immunoglobulins with variant Fc regions were assayed for their ADCC activity relative to a 4-4-20 immunoglobulin with a wild-type Fc region. These results are summarized in Table 11.

Please amend paragraph 00413 as follows:

[00413] ADCC assays were also carried out using the same protocol as previously described for the 4-4-20 antibody, however, the variant Fc regions were cloned into a humanized antibody (Ab4D5) which is specific for the human epidermal growth factor receptor 2 (HER2/neu). In this case, SK-BR-3 cells were used as the target cells that were opsonized with a HER2/neu antibody carrying a variant Fc region. HER2/neu is

endogenously ~~epxressed~~ **expressed** by the SK-BR-3 cells and therefore present on the surface these cells. FIG. 13 shows the ADCC activity of HER2/neu antibodies carrying variant Fc regions. Table 12 summarizes the results of ADCC activity of the mutants in the context of the HER2/neu antibody. Normalization was carried out by comparing the concentration of the mutant to the wildtype antibody required for a specific value of percent cell lysis.

Please amend paragraph 00423 as follows:

[00423] The following mutant screens were aimed at identifying additional sets of mutants that show improved binding to FcγRIIIA and reduced binding to FcγRIIB. Secondary screening of selected Fc variants was performed by ELISA followed by testing for ADCC in the 4-4-20 system. Mutants were than selected primarily based on their ability to mediate ADCC via 4-4-20 using Fluorescein coated SK-BR3 cells as targets and isolated PBMC from human donors as the effector cell population. Fc mutants that showed a relative increase in ADCC, *e.g.*, an ~~enhancedment~~ **enhancement** by a factor of 2 were than cloned into anti-HER2/neu or anti-CD20 chAbs and tested in an ADCC assay using the appropriate tumor cells as targets. The mutants were also analyzed by BIAcore and their relative K_{off} were determined.